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Diagnostic concordance of reporting lymphovascular invasion in breast cancer

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ABSTRACT

Aims: This study aims to assess the diagnostic agreement of lymphovascular invasion (LVI) in invasive breast cancer (BC).

Methods: Data on LVI were collected from the UK NHSBSP pathology external quality assurance (EQA) scheme database. 101 BCs assessed over 10-year period (2004-2014) were included. Cases were scored by an average of 600 pathologists. Three H&E stained slides from each case were reviewed by 3 pathologists and additional variables were evaluated.

Results: In the whole series, the overall kappa value was 0.4 (range 0.26 to 0.53). On review, LVI was detected in all 3 slides in 20 cases (20%), in 2 slides in 12 cases and in 1 of the 3 slides in 9 cases and was not seen in 60 cases. For concordance analysis, the first and last groups were used to represent cases with definite (LVI+) and absent LVI (LVI-) respectively. In the LVI+ group (n=20), the level of agreement ranged from 0.54 to 0.99 (median 0.86). In the LVI- group (n=60) the level of agreement ranged from 0.52 to 1.00 (median 0.93), with 44% of cases showing inter-observer concordance of >95%. There was a correlation between increasing number of involved lymphovascular spaces in the section and higher LVI reporting concordance. Some degree of retraction/fixation artefacts was observed in 35% of cases; this was associated with a lower concordance rate. **Conclusions**: The concordance of reporting LVI is variable. Cases without LVI and those with multiple involved vessels are likely to have the highest concordance and the highest detection rates.

INTRODUCTION

Lymphovascular invasion (LVI), defined by the presence of tumour cells within lymphovascular spaces, in breast cancer (BC) is identified morphologically by microscopic examination of the primary tumour, with or without endothelial specific markers. LVI is considered as a marker of metastatic potential and its prognostic value has been demonstrated by several independent studies (1-9). In a previous study of the Nottingham series (n=3812 cases) diagnosed by any of 4 pathologists in routine practice over 15-year period (2), we demonstrated that LVI is not only an independent prognostic variable in the whole series, and in the various prognostic subgroups, but also provides a risk equivalent to that provided by one higher stage category (e.g. pT1 to pT2) or from metastatic tumour in one or two lymph nodes compared to lymph node negative disease (2). Despite this, there remain concerns regarding its inclusion in staging systems (10) and in prognostic risk assessment tools (11, 12). This mainly stems from one or more of the following factors: 1) Variation in the frequency of LVI in the different studies. Although this is often perceived as a reflection of inconsistency of its detection among pathologists, this variation may be related to inherent differences in the study cohorts; for example, the range of LVI varies from 12% in grade 1 and small size tumours to 69% in tumours with high nodal stage in the same cohort (2). 2) The difficulty and subjectivity in identifying morphologically subtle LVI or considering intratumoural LVI (13). Although it is reported that immunohistochemistry (IHC) can assist in the identification of up to 20% of morphologically undetectable LVI on haematoxylin and eosin (H&E) stained sections, this does not seem to increase its prognostic significance (2). Most of these cases and those that are reported as “probable LVI” on H&E stained sections show an outcome intermediate between the positive and negative groups, akin to the

intermediate risk category of multigene prognostic assays (2, 14). 3). Being excluded from guideline recommendations and for practical reasons, IHC is not routinely used to assess LVI in most centres due to the perceived low reproducibility of identification of LVI (15). In a previous concordance study of 295 breast cancers included in the UK National Health Service Breast Screening Programme (NHSBSP) pathology interpretive external quality assurance (EQA) scheme, agreement in the assessment of LVI ranged from fair to moderate, with an overall kappa value of 0.4 (16). However, due to the microfocal nature of LVI and the limitation of the scheme methodology, we hypothesised that these figures were not representative of the actual concordance levels of LVI in BC. As the level of diagnostic concordance of any prognostic variable is important in determining its clinical utility, the present study aims to investigate further the diagnostic agreement of LVI in BC in detail, and to evaluate the reasons underlying the apparent low interobserver agreement, in an attempt to improve its clinical validity.

MATERIALS AND METHODS

Study cohort

This study is based on data obtained from the NHSBSP breast pathology EQA over a 10 year period (2005-2014). Description and details of the standard operating procedures have been published previously (15, 17, 18). In brief, over 65 sets (approximately 1 set per 10 participants) of 12 cases are circulated twice a year (over a three month period) to pathologists who report breast pathology in the UK (consultant pathologists with FRCPath or an equivalent degree with no restriction based on experience or number of breast specimens reported). Each case

comprises one freshly prepared haematoxylin and eosin (H&E) stained slide from a representative tumour tissue block of therapeutically surgically excised specimens (no biopsy material was included). No clinical details or immunohistochemistry results are provided. The cases are submitted by participants for use in the scheme. The slides are checked at the coordinating centre and reviewed by the organiser following preparation of the sections. Cases are then eliminated if section quality is too poor to interpret the histological appearances adequately, or if the key lesion is not adequately represented in all the sections cut. LVI is not a criterion for inclusion or exclusion of cases. A standard reporting form is completed by each participant for each case and this includes diagnostic classification of the lesion. For cancer cases, this includes the minimum standard data set information, as required by the UK NHSBSP and by the Royal College of Pathologists (19-21) (http://www.rcpath.org/publications-media), which includes LVI as one item. In the scheme, LVI is presently reported as present or absent with no probable/possible category. The scheme includes an average of 650 participants (range 602 to 749). The participating pathologist independently examines the slides and, for each case, completes a tick box proforma, now an online electronic process (http://www.nccbp.com/).

The level of agreement was assessed using κ statistics, as previously described (15, 22, 23). Values of κ range from 0 for chance agreement only to +1 for perfect agreement, with a negative value implying systematic disagreement. The range of κ is interpreted as follows: 0–0.20 = slight agreement; 0.21–0.40 = fair; 0.41–0.60 = moderate; 0.61–0.80 = substantial; and 0.81–1.00 = almost perfect agreement.

Histological review and analysis

During the time period of this study 120 invasive carcinoma cases were included in the scheme. The present analysis includes 101 BCs with LVI data recorded and available and where 3 H&E sections were available for review. Cases with incomplete data or missing slides were excluded (n=19). From each case, 3 H&E freshly stained slides (first, middle and last levels of average 70 sections from each tumour block) were reviewed by three pathologists (PP, AA and ER). The following features were scored: 1- number of slides showing definite LVI agreed by the reviewing pathologists. 2- number of involved lymphovascular spaces per case, counted as the number of images of LVI regardless of the likely number of vessels involved (i.e. tangential cut vessels; Figure 1). This has been scored as 0, 1, 2, 3, 4, 5 or more than 5. The number of foci of LVI was counted in the 3 slides and the highest number was recorded. 3- size of the largest intravascular tumour embolus. 4- location of LVI (peritumoural or away from the main tumour mass) and the distance from the tumour in mm. 5- presence of groups of tumour cells in spaces around the main tumour mass. 6- presence of erythrocytes and/or thrombus. 7- presence of adjacent lymphovascular channels of varying sizes. 8- presence of adjacent thick wall vessels. 9- presence and degree of shrinkage artefact on each slide. 10- presence and number of foci suspicious of LVI. 11- presence of DCIS. Foci that were considered not definite LVI were classified as negative following discussion between the three scorers. No immunohistochemical markers were assessed in this study.

RESULTS

This study included 101 BC that had been assessed by an average of 600 pathologists (range 477 to 672). LVI involvement was observed in all 3 slides in 20 cases, in 2 slides in 12, in 1 of the 3 slides in 9 cases and in none of the 3 in 60 cases (Table 1). Cases with LVI seen in all 3 slides were considered as showing definite LVI (LVI+) (n=20) whereas cases with no LVI in any of the 3 slides examined were considered as LVI negative (LVI-) (n=60). These 2 categories only (LVI+ and LVI-) were used to analyse concordance. Cases with LVI seen in only 1 or 2 of the 3 slides were excluded from the concordance analysis as it was considered more likely in this situation that some participants received levels for assessment where LVI was genuinely not present. Analysis of these cases with microfocal LVI may therefore not truly reflect agreement in assessment. Conversely, if LVI was present in all 3 levels examined, it was considered more likely that it was present in the sections in between (although not invariably the case) and these were included in the concordance investigation. Overall, the concordance rate of reporting LVI was >95% in 26 (25%) cases. Concordance between 75% and 95% was identified in 50 cases (50% of the total). The highest concordance rates were seen in the LVI- group, with 44% of cases having concordance of >95%. In the LVI- group, the 3 cases which showed concordance rates <65% featured significant retraction artefact throughout the tumour, making interpretation difficult (Figure 2).

In the LVI+ group, 86% of the participants (7814/9087 of the overall scores) diagnosed these 20 cases as LVI positive. On review, the 2 cases with concordance <65% showed that one of the 3 slides contained one (in one case) or two (in the other case) tiny foci of LVI only, which may indicate that some slides in between may be missing LVI.

There was a correlation between higher concordance rates and increasing number of involved vessels (p=0.001). The number of involved vessels varied from 1 (10%) to ≥6 images (40%). No statistical association was identified between of LVI concordance rates and size of the involved vessels, distance of the vessels from the tumour, presence of fibrous tracts, dilated vessels, or red blood cells or thrombosis within the involved vessels (p>0.05).

There was an association between histological grade of the tumour and reporting of LVI in both the LVI- and LVI+ subgroups. In the LVI- group, grade was inversely associated with LVI classification; in grade 1 tumours 94% of participants reported an absence of LVI compared to 90% in grade 2 and 82% in grade 3 tumours (p=0.011). In the LVI+ group, grade was positively associated with rates of reporting of LVI; the detection rate was 92% in grade 3 tumours compared to 78% in grade 2 cancers (p=0.039).

Certain histological tumour types showed high levels of concordance including tubular (100%), medullary (99%), adenoid cystic (96%), and classical lobular (95%) carcinomas. Three cases of invasive micropapillary carcinomas were included in this study and all showed LVI with a mean concordance rate of 91%. Other types showed lower concordance rates, including pleomorphic lobular carcinoma (65%), invasive papillary (68%) and a single case of glycogen rich carcinoma (64%). Mucinous and ductal NST lesions showed 85% and 81% concordance rates respectively.

Some degree of retraction/fixation artefacts was observed in 35% of cases, including the LVI+ (40%) and LVI- (32%) groups but this difference was not significant (p=0.587). Also no association between retraction artefacts and the presence of LVI

in the 1, 2 or 3 slides from each case was found. However, there was a correlation between the presence of retraction artefacts and lower concordance rates of reporting LVI in the whole cohort (p=0.009), and in the LVI- subgroup in which the mean concordance rate declined from 91% to 78% (p<0.001). In the LVI+ group the rate of reporting LVI increased from 79% to 89% when retraction artefacts were present but the difference was not significant (p=0.115).

There was a correlation between the number of involved vessels and the presence of LVI in more of the reviewed slides; all cases with ≥4 involved vessels were seen in the 20 cases in which LVI was seen in the 3 slides (p=0.044). Similar associations were found with the presence of dilated vascular space (p=0.048) and adjacent vascular channels of variable size (p=0.034).

The difference between scheme participants and scheme co-ordinators was statistically significant in the LVI+ group in which co-ordinators showed a higher detection rate of LVI than participants (p=0.008). No differences between scheme participants and co-ordinators was observed in the LVI- group or in the groups showing LVI in 1 or 2 of the 3 slides (p>0.05)

DISCUSSION

In the context of interobserver agreement studies, the UK EQA scheme provides a reflection of routine practice to a large extent, and the current study provide the largest sample size reported to date in terms of number of participants and invasive breast carcinomas. Although LVI is not a scored item of performance appraisal in the scheme, the current study indicates high levels of concordance. In a previous study of interobserver agreement utilising one slide per case prepared as glass slides

and/or digital images of scanned slides, LVI achieved higher level of concordance than histological grade, tubule formation or tumour lymphocytic infiltrate (24).

Of note, the current study highlights the microfocal nature of LVI and that only 20% of cases showed evidence of LVI in all 3 levels, representing a consistent presence in the tumour tissue, whilst 21% showed only focal and thus inconsistent LVI foci (i.e. seen in some but not all levels). The presence of LVI in some slides but not in others may explain the lower interobserver agreement in some studies involving multiple tissue levels. However, this study indicates that when there are 4 or more involved vessels present the likelihood that LVI will be seen in any section randomly cut from the tissue blocks and recognised by the pathologist as 'positive' is high. There is evidence that patients with tumours with extensive LVI have a shorter survival than those with focal, or without, LVI (25). In breast carcinomas with extensive LVI and therefore poor prognosis, LVI is likely to be more widely presented, detected in any section randomly cut and likely to be reported by the pathologist. Breast carcinomas with fewer foci of LVI have a lower chance of being reported as LVI positive, but these may not significantly affect the outcome of large cohort studies. This is in keeping with the association between LVI and outcome in different studies despite the variation in positivity rates in these studies.

This study emphasises the impact of methodology on the published concordance rate of such microfocal lesions. The possibility of different findings in other slides not examined in this study (i.e. the slides in between the 3 examined) cannot be excluded and this may have a detrimental impact on the concordance rate. Although in the LVI positive and negative groups we have found high concordance rates of LVI

detection and reporting, using a single slide in the EQA scheme, for instance utilising a digital imaging platform, may further improve consistency of LVI reporting.

This study also demonstrates a relationship between consistency of reporting LVI and features of the primary invasive tumour, including histological grade and type. Although some tumour types in this study are associated with low concordance rates, these tumours are rare; it is likely that the participant pathologists may pay more attention to the primary diagnosis of these rare invasive tumours than assessing prognostic markers in such EQA schemes. However, in routine practice, once an invasive diagnosis is made, we believe it is likely that pathologists will assess other prognostic variables, including LVI, more thoroughly.

In this EQA scheme cases are submitted randomly, being chosen from routine practice by participating pathologists. Thirty five % of cases showed some degree of retraction/fixation artefacts as assessed by the reviewing pathologists. There was an inverse association between the presence of retraction artefacts and concordance of LVI reporting; more LVI- tumours were reported as positive in the presence of retraction artefact. In the LVI+ group the rate of reporting LVI also increased by 10% when retraction artefact was present but no IHC was available to confirm whether this increase was related to the presence of artefactual shrinkage or genuine LVI. The presence of red blood cells in the involved vessels, number of tumour cells within the vessels, and location of the vessels appeared of limited impact on consistency of LVI reporting.

In conclusion, these results demonstrate a high level of consistency of reporting LVI in breast cancer. Limitations in the methodology of concordance studies may

underestimate performance in routine practice. Education, training and guidance are expected to improve consistency, and to maintain the high level of performance.

Take home messages

-The concordance of reporting lymphovascular invasion in breast cancer is variable.

-There is a correlation between increasing number of involved lymphovascular spaces in the section and higher LVI reporting concordance.

-Cases without LVI and those with multiple involved vessels are likely to have the highest concordance and the highest detection rates.

-When LVI is present in the slide, the median level of agreement is 0.86 and when it is absent the median level of agreement is 0.93.

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Licence for Publication

Competing Interest

None declared.

Contributorship

All authors contributed to this study and all approved the final manuscript

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Not applicable

Data sharing statement

Data are available upon request and at the discretion of the authors.

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Tables

Table 1: Mean, median and range of frequency of reporting (%) LVI in the whole series (n=101), LVI positive (n=20) and LVI negative (n=60) cases and those with LVI seen only in 1 or 2 of the 3 slides (n=21) by all participants and by the scheme co-ordinators.

	Mean	**Median**	**Range**
Whole series			
Pathologists	0.83	0.88	0.50-1.00
Co-ordinators	0.86	0.89	0.50-1.00
LVI definite (in all 3 slides)			
Pathologists	0.83	0.86	0.54-0.99
Co-ordinators	0.89	0.95	0.63-1.00
LVI negative (in all 3 slides)			
Pathologists	0.88	0.93	0.52-1.00
Co-ordinators	0.88	0.90	0.50-1.00
LVI seen in one slide			
Pathologists	0.73	0.65	0.61-0.95
Co-ordinators	0.83	0.86	0.65-1.00
LVI seen in two slides			
Pathologists	0.69	0.65	0.51-0.92
Co-ordinators	0.73	0.71	0.50-0.95

Figure legends

Figure 1: A case of breast cancer showing clear lymphovascular invasion adjacent to the invasive tumour and involving vascular space with tangential cutting. In this study this was counted as 3 images of lymphovascular invasion.

Figure 2: A case of breast cancer with retraction artefact making assessment of lymphovascular invasion difficult due to similarity to DCIS with shrinkage artefact